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Coordinated morphogenesis of neurons and glia

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summary

Glia adopt remarkable shapes that are tightly coordinated with the morphologies of their neuronal partners. Glia and neurons exhibit coordinated morphological changes on the time scale of minutes and on size scales ranging from nanometers to hundreds of microns. Here, we review recent studies that reveal the highly dynamic, localized morphological changes of mammalian neuron-glia contacts. We then explore the power of *Drosophila* and *C. elegans* models to study coordinated changes at defined neuron-glia contacts, highlighting the use of innovative genetic and imaging tools to uncover the molecular mechanisms responsible for coordinated morphogenesis of neurons and glia.

Introduction

Glia have been renowned for their exquisite morphology since long before their functions were known. Now, the functions of glia are increasingly well understood (see reviews [1,2]), yet glial morphogenesis remains as mysterious as ever. Astrocytes, once called “spider cells,” extend innumerable radiating processes that wrap synapses with puzzle-piece-like precision[3-6]. How do glia attain their remarkable shapes, and how are they coordinated with neuronal morphologies? Here, we review recent striking examples of coordinated neuron-glia morphogenesis in mammals. We then highlight the power of simple model organisms such as *Drosophila* and *C. elegans* to address these questions.

The scope of the problem: Highly dynamic and localized morphological changes

The intimate associations between glia and synapses exhibit highly dynamic morphological changes on the order of minutes[7-11]. Landmark studies using time-lapse confocal imaging of rodent brain slices revealed that post-synaptic dendritic spines and astrocytic processes do not change shape in perfect register, yet generally grow or shrink together over time[7,9].Remarkably, this coordinated growth is achieved even though glia-spine interactions undergo rapid structural changes, the extent of glial coverage of spines varies, and astrocytic processes tend to exhibit even greater motility than their dendritic spine counterparts[7].

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Two recent, complementary studies provided evidence for a mechanism that may help to explain the coordinated growth of astrocytic processes and dendritic spines. Perez-Alvarez et al. and Bernardinelli et al. examined coordinated morphological changes of astrocytic processes and dendritic spines in response to patterns of neuronal activity that increase the size of dendritic spines[12-14]. By observing single synapses, these groups found that stimulation of the presynaptic neuron also triggers a rapid, transient increase in the motility of the astrocytic processes associated with the dendritic spine (Fig. 1A, top). This effect is mediated by metabotropic glutamate receptors on astrocytes, which are bound by glutamate released by the presynaptic neuron upon stimulation. Activated receptors initiate intracellular calcium transients in astrocytes that are both necessary and sufficient for increased process motility. This spike in motility tends to expand astrocytic coverage of spines, such that subsequent stabilization of astrocytic processes (within ~30 min) produces a sustained increase in the extent of astrocytic wrapping of spines. These coordinated morphological changes in astrocytes and spines were shown to affect neural function[12,13]. Interestingly, similar changes were also seen following sensory stimulation *in vivo*, consistent with previous EM studies[13,15].

Not only are these morphological changes highly dynamic over time, but they are also exquisitely restricted in space - even to the level of specific synapses. Photoactivation of astrocytic calcium signaling at a single synapse causes changes in astrocytic process motility at that synapse, but does not affect nearby glia-spine contacts on the same dendrite[12]. This suggests that astrocytes can respond to the unique demands of each synapse, which is especially remarkable given that a single astrocyte may contact nearly 100,000 synapses in rodents and up to two million synapses in humans[16].

An impressive anatomical study recently underscored the idea that glia exhibit highly localized morphological patterning that is coordinated with their local neuronal environment. Wang et al. demonstrated that a single Müller glial cell that spans the entire thickness of the retina (~200 μm in adult mice) adopts strikingly different morphologies at each retinal layer (Fig. 1B, left) [17]. Its glial process courses in thin sheets through the outer nuclear layer but becomes wildly branched in the synapse-rich environment of the inner plexiform layer (~50 μm each layer).In fact, the specificity of glial shape is so tailored to the local environment that Müller glia even elaborate distinct structures at different sublaminar positions within the inner plexiform layer. Such localized morphological differences were also noted in a recent characterization of radial glia-like stem cells in the dentate gyrus[18]. These cells extend thin, twisting processes through the granule cell layer (~30 μm) and then arborize abruptly upon encountering the molecular layer, where they ensheathe synapses.

While these studies demonstrate that glial shapes are precisely patterned at the level of tens to hundreds of microns, another recent study suggests that astrocytic processes are also patterned at the submicron scale. Pannasch et al. found that glial wrapping is actively restricted to regions of the spine adjacent to the synapse[19]. Loss of the gap junction protein Connexin 30 (Cx30) causes inappropriate invasion of astrocytic processes into the synaptic cleft, promoting increased astrocytic uptake of glutamate and thus reducing neurotransmission. An elegant combination of pharmacological and genetic manipulations

showed that this morphological intrusion was regulated not by the channel-forming properties of Cx30, but rather by signaling activity mediated through its intracellular region. These results raise the intriguing possibility that such fine-scale morphological changes might be actively regulated to modulate synaptic transmission.

These examples highlight the scope of the challenge in understanding coordinated neuron-glia morphogenesis: structural changes occur on the time scale of minutes, with highly specialized patterning that spans from the ultrastructural level to the tissue level. Additionally, advances in single-cell transcriptomics have revealed previously unrecognized heterogeneity among glia[20-22], raising the possibility of another dimension of complexity: specific subtypes of glia may preferentially coordinate with synapses from different types of neurons. Ideally, single glia-synapse contacts between defined neuronal and glial partners would be directly observed in intact, living animals, but these approaches are technically challenging amid the complexity of the mammalian nervous system. In contrast, such approaches have proven more feasible in invertebrate model organisms. The remainder of this review will focus on the use of these powerful models to understand coordinated neuron-glia morphogenesis.

The power of invertebrate systems

Simple anatomy, sophisticated genetics

The highly simplified nervous systems of invertebrates make these organisms tremendously powerful models for studying cooperative changes in cell shape. In addition to their simple anatomy and rapid developmental timeline, *Drosophila melanogaster* and *C. elegans* offer an impressive array of tools for facile genetic manipulation and unbiased forward genetic screens.

Drosophila has been an exceptionally strong model for probing glial biology, as the fly nervous system contains ramified glia with clear parallels to mammalian astrocytes. Detailed characterization of astrocyte morphologies and synaptic structures during *Drosophila* metamorphosis revealed that astrocytes invade the neuropil concomitant with synaptogenesis[23]. Thus, fine-scale changes in neuronal shape occur coordinately with large-scale morphogenetic changes in glia. Moreover, electron microscopy and fluorescence imaging of synapses and astrocytic processes in the larval ventral nerve cord have established that, as in mammals, astrocytes associate closely with synaptic structures and respond to local synaptic cues, although they do not ensheathe individual synapses[24]. These close associations are driven by astrocytic invasion of the neuropil. Analysis of candidate mutants and cell-specific rescue experiments demonstrated that this invasion requires activation of the *Drosophila* FGF receptor on astrocytes, most likely in response to neuronally-derived FGFs[24]. This suggests that glial morphologies can be specified by the neurons they contact.

Although these studies clearly highlight the power of *Drosophila*, this system has its drawbacks: with an estimated 250,000 neurons and 25,000 glia, using *Drosophila* to visualize asingle, defined cell-cell contact at high resolution is still technically difficult. Limitations on live imaging across development and the time-consuming nature of unbiased

forward genetic screens further restrict studies of cooperative glia-synapse morphogenesis in *Drosophila*[25].

C. elegans offers a complementary model system to overcome these challenges. Each cell in the nematode is uniquely identifiable and derived from a known lineage, such that the nervous system of the adult hermaphrodite consists of exactly 302 neurons, 50 neural-derived glia, and 6 mesoderm-derived "glial-related" cells. Remarkably, each of these cells adopts a stereotyped shape and reproducible set of cell-cell contacts. All of these neuron-glia contacts have been catalogued and, as described below, can be grouped into three main classes. Most importantly, this means that a single, defined neuron-glia contact can be revisited across many individuals in wild-type or mutant backgrounds, and can be visualized and manipulated in live animals using a well-developed toolbox of cell-specific promoters to target the expression of transgenes to individual cells. This capacity for labeling single contacts is especially powerful given the transparency and small size of the nematode, which facilitate time-lapse imaging across development and super-resolution imaging of live, anesthetized animals (see Box). Together with the ease of unbiased forward genetic screens, these tools make *C. elegans* a tremendously potent model system for studying questions of coordinated neuron-glia morphogenesis. Yet this system has its own drawbacks: in contrast to *Drosophila*, glia in *C. elegans* are not clearly analogous to specific types of mammalian glia, such as astrocytes or oligodendrocytes. Nevertheless, parallels are emerging between the functions and molecular determinants of *C. elegans* and mammalian glia, as illustrated by the studies described below.

Overview of neuron-glia contacts in C. elegans

Prodigious efforts to fully reconstruct the architecture of the nervous system by electron microscopy have led to an unprecedented map of *C. elegans* neuron-glia contacts at the single-cell level[26-28]. All glia in this organism associate with sensory neurons to create sense organs[29,30]. Most of these are located in the animal's head and contain two glial cells of different types, called the sheath and the socket. Each glial cell extends an unbranched process that fasciculates with the dendrites of its neuronal partners and terminates at the tip of the nose.

Three classes of neuron-glia contacts have been described in *C. elegans*. The first is comprised of contacts between the distal-most region of the sheath glial process and the ciliated receptive endings (REs) of the sensory dendrites of its neuronal partners (Fig. 2A,i). The glial cell ensheathes these dendrites and forms epithelial-like junctions around them; the sheath also intimately wraps a subset of ciliated REs that are embedded within it. These ciliated REs are functionally analogous to dendritic spines; both are highly specialized compartments that contain machinery required to sense cues in the extracellular environment – be that the animal's surroundings or the synaptic cleft[31]. In each case, the tightly interlocking shapes of the two cells allude to precise coordination of neuronal and glial morphologies.

The second class of neuron-glia contacts consists of axon-glia interactions in which a sheath glial cell envelops axons in the central neuropil and promotes synaptogenesis via secreted cues (Fig. 2A,ii) [32-34].Along with the previously described glia-RE contacts, these have

been the focus of all existing studies on glia in the nematode. However, a third class of neuron-glia contact has been observed by electron microscopy. These contacts also involve glia and dendritic REs, but they are more reminiscent of adhesion between mammalian glia and dendritic spines because they lack the epithelial-like junctions described above [26,28]. In one unique example, the “BAG” neuron RE precisely and reproducibly wraps a protrusion extending from its glial partner (Fig. 2A,iii). This raises several questions: what determines the exquisite morphology of a neuron-glia contact? How do the cells communicate to coordinate changes in cell shape? Is coordinated morphogenesis driven by the neuron, the glial cell, or both?

Coordinated morphogenesis of single neuron-glia contacts in C. elegans

Several studies have demonstrated that *C. elegans* neurons and glia undergo coordinated changes in morphology at sites of neuron-glia contact. First, in studies of glia-axon interactions, unbiased forward genetic screens have shown that synapses in the central neuropil are initially positioned by glia[32]. These synaptic positions are then maintained by epidermal signals that coordinate glial morphogenesis with larval growth [32,34]. Second, during embryonic development, morphogenesis of the sheath glial process and neuronal dendrites are coordinated, such that mutants with failures in dendrite extension show equivalent defects in glial process morphology[35]. Similarly, mutants that disrupt glial development also lead to defects in dendrite extension[33]. These effects are seen at size scales on the order of 100 μm (Fig. 1B, right).

Glia also play a role in the fine sculpting of REs at the scale of a few microns, comparable to the size of a mammalian synapse. When animals enter into an alternative life stage called dauer, some neurons undergo extensive structural remodeling over a period of about 6h[36].The sheath glial cell and the RE of one of its neuronal partners remodel in concert, expanding to encompass more than half the circumference of the head (Fig. 1A, bottom) [37-39]. In this case, the glial cell physically constrains the expansion of the neuronal RE, though glial remodeling occurs independently of neuronal changes. Further, in studies using standard (i.e. non-dauer) growth conditions, laser ablation of single glial cells and cell-specific inhibition of secretion in glia revealed that glia are also required to maintain the shape of neuronal REs embedded within them[40,41]. For example, RNAi-mediated knockdown of glial-enriched genes demonstrated that the transcription factor PROS-1/ Prospero functions in glia to regulate the shape of associated dendritic REs[42].

Recent work by Singhvi and colleagues demonstrates that interactions at RE-glia contacts can be uniquely tailored to the identity of the neuron[41]. Candidate screens for morphology mutants identified a potassium-chloride co-transporter, KCC-3, that is expressed by the sheath glial cell. Remarkably, KCC-3 localizes specifically to the region of glial membrane adjacent to just one of the 12 REs ensheathed by the glial cell (Fig. 2B). Mutations in this co-transporter could be rescued by supplying high exogenous levels of potassium and chloride ions, implying that the co-transporter modulates the morphology of this neuronal RE by regulating ion concentrations in the interstitial space. This suggests the sheath glial cell can generate specialized subdomains tailored for particular neuronal partners. Future work will need to address how this specificity is achieved: how do neurons and glia

recognize the identity of their partners? Do mammalian astrocytes similarly discriminate among dendritic spines from different neuron types?

In each of the examples discussed above, glia exert control over neuronal shape. Preliminary evidence suggests that neuronal cues can also regulate the fine-scale structure of glia in *C. elegans*[43,44]. For example, electron micrographs of mutants whose sensory neurons lack dendritic spine-like ciliary endings reveal changes in the fine-scale morphology of the sheath glial cell[44,45]. While the identities of neuronal signals remain enigmatic, the power and ease of unbiased genetic screens in *C. elegans* promise to uncover the molecular mechanisms by which neurons drive cell shape changes in glia.

Conclusion and Perspectives

Studies in vertebrate systems have described tightly interlocking neuronal and glial morphologies and have revealed striking examples of the coordinated morphogenesis of these cells. However, the detailed structures of glia-synapse contacts are highly heterogeneous and difficult to study at high temporal and spatial resolution in the mammalian brain. In contrast, an expanding toolbox in *Drosophila* and *C. elegans* will allow single defined neuron-glia contacts to be observed during development, throughout life, and in response to environmental and genetic perturbations. This experimental framework provides a powerful means to uncover the cellular and molecular mechanisms that govern coordinated neuron-glia morphogenesis.

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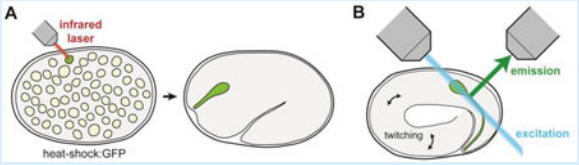
Box 1

Technological innovations for visualizing coordinated neuron-glia morphogenesis in *C. elegans*



While single, defined neuron-glia contacts can be readily visualized in the mature C. elegans nervous system, a major remaining challenge for studying coordinated neuron-glia morphogenesis is to see these structures in the developing embryo. This challenge stems from two technical impediments. First, cell-specific markers from the mature adult are often not active in the early embryo, making it difficult to label single neuron-glia attachments. Second, rapid embryonic twitching movements – combined with the drug-impermeable eggshell that prevents the use of paralytics – necessitate high temporal resolution when imaging live embryos.

Recently, major inroads have been made to address each of these hurdles. First, single-cell heat-shock induction, schematized in part A of this figure, as well as photoconversion of fluorescent proteins, have been used to achieve cell-specific labeling in the embryo[35,46]. Second, advances in light microscopy – especially the invention of Dual-View Selective Plane Illumination Microscopy (diSPIM), schematized in part B of this figure – have made it possible to image cells in the developing embryo at high spatial and temporal resolution over the entirety of embryogenesis[47,48]. This allows acquisition of clear images through the complete volume of the embryo despite embryonic twitching movements. In addition to these advances in image acquisition, novel post-processing techniques have made it feasible to track single cell movements within the moving embryo[49,50]. Armed with these advances, efforts are now underway to generate large-scale atlases of all cellular positions throughout embryogenesis[49]. In conjunction with recent advances in EM of defined embryonic stages[51], these techniques will facilitate additional studies of neuron-glia contact formation by revealing how neurons and glia move relative to one another in the developing embryo.

Highlights

- Glial and neuronal morphogenesis is coordinated at size scales from <1μm to 100 μm.
- Presynaptic signals help to coordinate changes in dendritic spines and glial processes.
- C. elegans has highly stereotyped contacts between defined neurons and glia.
- *C. elegans* glia can actively shape their neuronal partners.

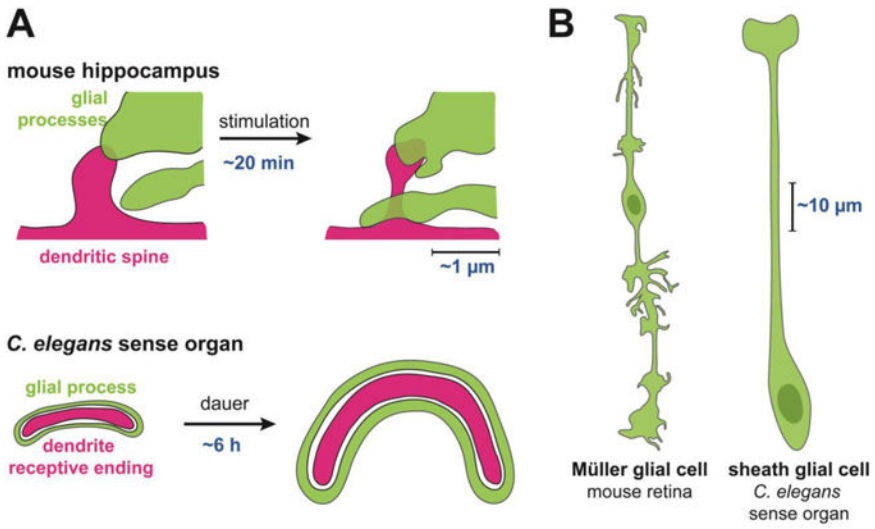


Figure 1. Glia and neurons undergo dynamic, localized morphological changes in mammals and invertebrates

(A) Glial processes (green) exhibit coordinated changes with dendritic spines or receptive endings (red). These occur over minutes to hours on the scale of microns in response to experimental stimulation or environmental conditions. (B) A single glial cell can exhibit highly localized morphological patterning that is coordinated with its local neuronal environment, on the scale of tens to hundreds of microns.

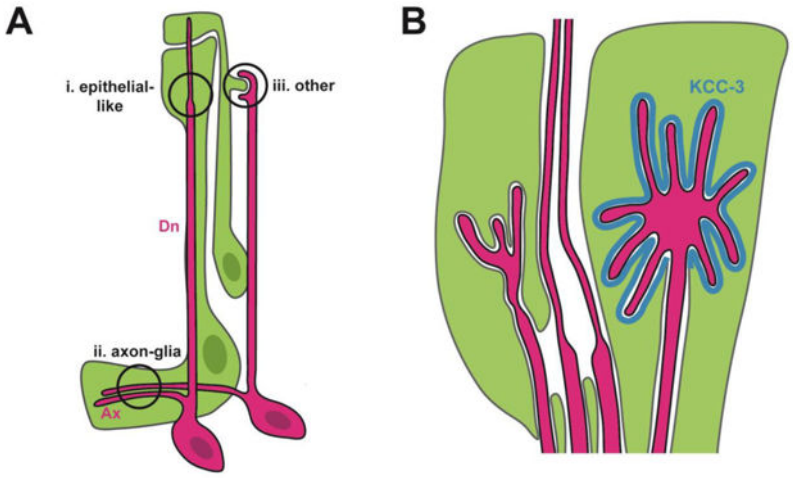


Figure 2. *C. elegans* as a model for coordinated neuron-glia morphogenesis

(A) Three main classes of neuron-glia contacts in *C. elegans*. Neurons, red; glia, green. Ax, axons; Dn, dendrite. (B) The glial cell can create specialized subdomains tailored for specific neuronal partners, as evidenced by the localization of the KCC-3 ion co-transporter (blue) in the glial cell membrane exclusively adjacent to a single dendrite receptive ending.